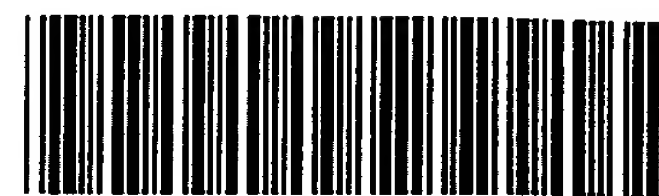


#11

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RAW SEQUENCE LISTING

DATE: 04/26/2002

PATENT APPLICATION: US/09/697,186A

TIME: 11:52:32

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3 <110> APPLICANT: YOKOYAMA, SIGEYUKI
 4 HOSEKI, JUN
 5 YANO, TAKATO
 6 KOYAMA, YOSHINORI
 7 KURAMITSU, SEIKI
 8 KAGAMIYAMA, HIROYUKI
 10 <120> TITLE OF INVENTION: MUTANT KANAMYCIN NUCLEOTIDYLTRANSFERASE AND A METHOD
 11 OF SCREENING THERMOPHILIC BACTERIA USING THE SAME
 13 <130> FILE REFERENCE: 04853.0048-00000
 15 <140> CURRENT APPLICATION NUMBER: 09/697,186A
 C--> 16 <141> CURRENT FILING DATE: 2002-04-11
 18 <150> PRIOR APPLICATION NUMBER: JP 309616/1999
 19 <151> PRIOR FILING DATE: 1999-10-29
 21 <160> NUMBER OF SEQ ID NOS: 20
 23 <170> SOFTWARE: PatentIn Ver. 2.1
 25 <210> SEQ ID NO: 1
 26 <211> LENGTH: 253
 27 <212> TYPE: PRT
 28 <213> ORGANISM: Artificial Sequence
 30 <220> FEATURE:
 31 <223> OTHER INFORMATION: Description of Artificial Sequence: Mutant enzyme
 32 obtained by introduction of point mutation into
 33 wild type KNT gene of Staphylococcus aureus and
 34 its expression
 36 <400> SEQUENCE: 1
 37 Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val
 38 1 5 10 15
 40 His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
 41 20 25 30
 43 Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
 44 35 40 45
 46 Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Glu Ala Glu Phe
 47 50 55 60
 49 Ser His Glu Trp Thr Thr Gly Glu Trp Lys Val Glu Val Asn Phe Tyr
 50 65 70 75 80
 52 Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Gln Val Glu Ser Asp Trp
 53 85 90 95
 55 Pro Leu Thr His Gly Gln Phe Phe Ser Ile Leu Pro Ile Tyr Asp Ser
 56 100 105 110
 58 Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala
 59 115 120 125
 61 Gln Lys Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe
 62 130 135 140

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64 Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr
65 145                      150                      155                      160
67 Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu
68                      165                      170                      175
70 Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu
71                      180                      185                      190
73 Thr Glu Ala Val Lys Gln Ser Asp Leu Pro Ser Gly Tyr Asp His Leu
74                      195                      200                      205
76 Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu
77                      210                      215                      220
79 Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg
80 225                      230                      235                      240
82 His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe
83                      245                      250
86 <210> SEQ ID NO: 2
87 <211> LENGTH: 253
88 <212> TYPE: PRT
89 <213> ORGANISM: Artificial Sequence
91 <220> FEATURE:
92 <223> OTHER INFORMATION: Description of Artificial Sequence: Mutant enzyme
93     obtained by introduction of point mutation into
94     wild type KNT gene of Staphylococcus aureus and
95     its expression
97 <400> SEQUENCE: 2
98 Met Lys Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val
99  1                      5                      10                      15
101 His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
102                      20                      25                      30
104 Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
105                      35                      40                      45
107 Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Gly Ala Glu Phe
108                      50                      55                      60
110 Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Tyr
111 65                      70                      75                      80
113 Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Ser Asp Trp
114                      85                      90                      95
116 Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro
117                      100                     105                     110
119 Gly Gly Tyr Phe Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala
120                      115                     120                     125
122 Gln Lys Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe
123                      130                     135                     140
125 Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr
126 145                      150                      155                      160
128 Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu
129                      165                      170                      175
131 Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu
132                      180                      185                      190
134 Thr Glu Ala Val Lys Gln Pro Asp Leu Pro Ser Gly Tyr Asp His Leu

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135          195          200          205
137 Cys Gln Leu Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu
138      210          215          220
140 Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg
141 225          230          235          240
143 His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe
144          245          250
147 <210> SEQ ID NO: 3
148 <211> LENGTH: 253
149 <212> TYPE: PRT
150 <213> ORGANISM: Artificial Sequence
152 <220> FEATURE:
153 <223> OTHER INFORMATION: Description of Artificial Sequence: Mutant enzyme
154      obtained by introduction of point mutation into
155      wild type KNT gene of Staphylococcus aureus and
156      its expression
158 <400> SEQUENCE: 3
159 Met Lys Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val
160  1          5          10          15
162 His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
163      20          25          30
165 Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
166      35          40          45
168 Ser Asp Ile Glu Met Met Cys Val Leu Ser Thr Glu Gly Val Glu Phe
169      50          55          60
171 Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Tyr
172  65          70          75          80
174 Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Pro Asp Trp
175      85          90          95
177 Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro
178      100         105         110
180 Gly Gly Tyr Phe Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala
181      115         120         125
183 Gln Lys Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe
184      130         135         140
186 Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr
187 145         150         155         160
189 Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu
190      165         170         175
192 Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu
193      180         185         190
195 Thr Glu Ala Val Lys Gln Pro Asp Leu Pro Pro Gly Tyr Val Gln Leu
196      195         200         205
198 Cys Gln Leu Val Met Ser Gly Gln Leu Ser Asp Pro Glu Lys Leu Leu
199      210         215         220
201 Glu Ser Leu Glu Asn Phe Trp Asn Gly Val Gln Glu Trp Ala Glu Arg
202 225         230         235         240
204 His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe
205          245         250

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Input Set : A:\48530048.app

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210 <212> TYPE: DNA
211 <213> ORGANISM: Artificial Sequence
213 <220> FEATURE:
214 <223> OTHER INFORMATION: Description of Artificial Sequence: Primer for
215     PCR amplification
217 <400> SEQUENCE: 4
218 gactgtacgg gtacccgttg acggcggata tggta 35
221 <210> SEQ ID NO: 5
222 <211> LENGTH: 35
223 <212> TYPE: DNA
224 <213> ORGANISM: Artificial Sequence
226 <220> FEATURE:
227 <223> OTHER INFORMATION: Description of Artificial Sequence: Primer for
228     PCR amplification
230 <400> SEQUENCE: 5
231 gactgtacgc tgcagcgtaa ccaacatgat taaca 35
234 <210> SEQ ID NO: 6
235 <211> LENGTH: 35
236 <212> TYPE: DNA
237 <213> ORGANISM: Artificial Sequence
239 <220> FEATURE:
240 <223> OTHER INFORMATION: Description of Artificial Sequence: Primer for
241     PCR amplification
243 <400> SEQUENCE: 6
244 gactgtacgg aattcgagct cgagcaaatac taaaa 35
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248 <211> LENGTH: 35
249 <212> TYPE: DNA
250 <213> ORGANISM: Artificial Sequence
252 <220> FEATURE:
253 <223> OTHER INFORMATION: Description of Artificial Sequence: Primer for
254     subcloning of WT
256 <400> SEQUENCE: 7
257 gactgtacgc atatgaatgg accaataata atgac 35
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262 <212> TYPE: DNA
263 <213> ORGANISM: Artificial Sequence
265 <220> FEATURE:
266 <223> OTHER INFORMATION: Description of Artificial Sequence: Primer for
267     subcloning of KT3-11 and HTK
269 <400> SEQUENCE: 8
270 gactgtacgc atatgaaagg accaataata atgac 35
273 <210> SEQ ID NO: 9
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275 <212> TYPE: DNA
276 <213> ORGANISM: Artificial Sequence

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RAW SEQUENCE LISTING

PATENT APPLICATION: US/09/697,186A

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278 <220> FEATURE:

279 <223> OTHER INFORMATION: Description of Artificial Sequence: Primer for subcloning

281 <400> SEQUENCE: 9

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288 <213> ORGANISM: Staphylococcus aureus

290 <220> FEATURE:

291 <221> NAME/KEY: CDS

292 <222> LOCATION: (1)..(759)

294 <400> SEQUENCE: 10

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297 1 5 10 15

299 cat gaa att aag gaa cga ata ttg gat aaa tat ggg gat gat gtt aag 96

300 His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys

301 20 25 30

303 gct att ggt gtt tat ggc tct ctt ggt cgt cag act gat ggg ccc tat 144

304 Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr

305 35 40 45

307 tcg gat att gag atg atg tgt gtc atg tca aca gag gaa gca gag ttc 192

308 Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Glu Ala Glu Phe

309 50 55 60

311 agc cat gaa tgg aca acc ggt gag tgg aag gtg gaa gtg aat ttt gat 240

312 Ser His Glu Trp Thr Thr Gly Glu Trp Lys Val Glu Val Asn Phe Asp

313 65 70 75 80

315 agc gaa gag att cta cta gat tat gca tct cag gtg gaa tca gat tgg 288

316 Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Gln Val Glu Ser Asp Trp

317 85 90 95

319 ccg ctt aca cat ggt caa ttt ttc tct att ttg ccg att tat gat tca 336

320 Pro Leu Thr His Gly Gln Phe Phe Ser Ile Leu Pro Ile Tyr Asp Ser

321 100 105 110

323 ggt gga tac tta gag aaa gtg tat caa act gct aaa tcg gta gaa gcc 384

324 Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala

325 115 120 125

327 caa acg ttc cac gat gcg att tgt gcc ctt atc gta gaa gag ctg ttt 432

328 Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe

329 130 135 140

331 gaa tat gca ggc aaa tgg cgt aat att cgt gtg caa gga ccg aca aca 480

332 Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr

333 145 150 155 160

335 ttt cta cca tcc ttg act gta cag gta gca atg gca ggt gcc atg ttg 528

336 Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu

337 165 170 175

339 att ggt ctg cat cat cgc atc tgt tat acg acg agc gct tcg gtc tta 576

340 Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu

341 180 185 190

343 act gaa gca gtt aag caa tca gat ctt cct tca ggt tat gac cat ctg 624

VERIFICATION SUMMARY

PATENT APPLICATION: US/09/697,186A

DATE: 04/26/2002

TIME: 11:52:33

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